

## CHONDROGENESIS OF BONE MARROW AND PERIPHERAL BLOOD DERIVED ADULT HUMAN MESENCHYMAL STEM CELLS

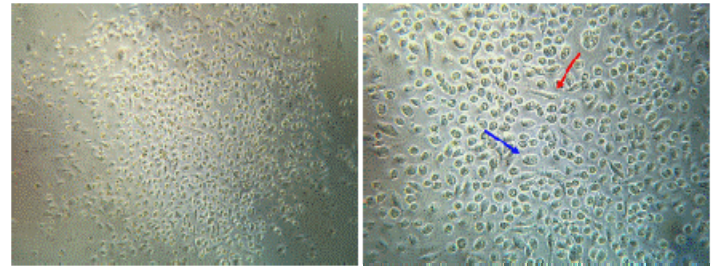
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**INTRODUCTION:** Although mesenchymal stem cells (MSCs) isolation from a number of tissue sources have been described, very few literatures have reported successful isolation of adult MSCs from peripheral blood or its chondrogenic differentiation for clinical applications. The objective of this study is to isolate MSCs derived from both human bone marrow and peripheral blood and to compare their potential to undergo chondrogenesis.

**METHODS:** Bone marrow (BM) and peripheral blood (PB) samples were collected into blood collection tubes containing lithium heparin (2-4mls). Mononuclear cells were separated from both types of samples using Ficoll-Paque PLUS by centrifugation and suspended in cell culture medium before being plated onto tissue culture flasks. Suspended cells were subsequently removed after 5 days of culture, and adherent cells were left to grow. Cells were sub-cultured (2–5 passages) prior to further cellular analyses and differentiation experiments. Chondrogenic pellets were harvested after 4-5 weeks in culture. To assess chondrogenesis, alcian blue and safranin-O were used to determine whether cartilage matrix proteoglycan was expressed. Chondrogenesis was also quantified by the amount of sulphated glycosaminoglycan (S-GAG) production measured using 1,9-dimethylmethylene blue (DMMB) assay.

**RESULTS:** Based on our results, we were able to establish techniques for isolation of MSCs from BM and PB. The presence of surface marker proteins CD44, CD105, CD166 and the absence of CD34 in these cells (confirmed using flow-cytometry) indicate the high likelihood of successful mesenchymal stem cell isolation. Histological examination revealed significant cellular expressions of proteoglycans and glycosaminoglycans indicating successful induction of chondrogenesis in our isolated MSCs.



*Fig1. Human blood derived-MSCs: the predominant cells consist of fibroblast-like cells.*

*Table1. Flow cytometry analysis: Immune phenotype of culture-expanded MSCs..*

| Antigen    | CD number | Expression |
|------------|-----------|------------|
| HCAM-1     | CD44      | Positive   |
| Endoglin   | CD105     | Positive   |
| SB10/ALCAM | CD166     | Positive   |
| HCAM-1     | CD34      | Negative   |

**DISCUSSION & CONCLUSIONS:** *In vitro* induction of chondrogenesis has been demonstrated in both bone marrow and peripheral blood-derived MSCs using 3-dimensional scaffold producing comparable cellular expressions. MSCs which are easily isolated (and less painfully harvested) from peripheral blood as compared to bone marrow provides a superior alternative source for MSCs for future clinical application (in this case for cartilage repair).

**REFERENCES:** Heng B. C., Cao T. and Lee, E. H. (2004) Stem cells **22**, 1152–1167. Hirsch, M. S., Lunsford L. E., Trinkaus-Randall, et al (1997) Dev. Dyn. **210**, 249–63.

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